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Synthesis and Antibacterial Screening of Polysubstituted Alkene Derivatives

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A mini combinatorial library of bi- and tri-substituted alkenes was synthesized and screened for antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeroginosa*. The library of substituted alkenes was obtained by nucleophilic condensation of some carbonyl compounds with a set of active methylene reagents under base catalysis. Screening of the pooled compounds for antibacterial activity and subsequent identification of the lead compound was done by two dimensional deconvolution analysis. The pure lead compound, 1-naphthyl-2-(2-carboxy 3-nitrophenyl) ethene, was synthesized and characterized using TLC, IR, ^{1}H NMR and elemental analysis.

Key Words: Antibacterial, Combinatorial, Polysubstituted alkene, Synthesis, Screening.

INTRODUCTION

Combinatorial chemistry is a modern technique for the synthesis of a very large number of structurally diverse molecules for biological evaluation in a time and resource effective manner. Combinatorial synthesis can be achieved either in solution or in solid-phase. Though solution phase multi-step synthesis involves a purification step after each step, its main advantage is its wide acceptability for a variety of substrates, reagents and practically limitless reaction conditions[1–3].

Bacterial cell wall inhibitory activity has been shown by some vinylbenzene derivatives[4]. Some alkenyl derivatives of heteroaryl molecules have been demonstrated to have antibacterial activity[5]. Cyclohexene derivatives have been shown to affect bacterial cell wall integrity through multiple mechanisms[6, 7]. Some nitro derivatives of heteroaryl, azomethine or vinyl compounds have been reported to have broad spectrum antibacterial activity[8]. Few polysubstituted alkene derivatives from natural origin have been identified to possess significant antimicrobial properties[9]. Considering the analogy with these structural features of some reported antibacterial compounds, a mini library of di- and trisubstituted ethene derivatives was synthesized and screened with a combinatorial approach employing reported methods for synthesis[10].

EXPERIMENTAL

Phenyl acetic acid, 2,4-dinitro phenylacetic acid, naphthyl acetic acid and acetophenone were employed as active methylene reagents for condensation with

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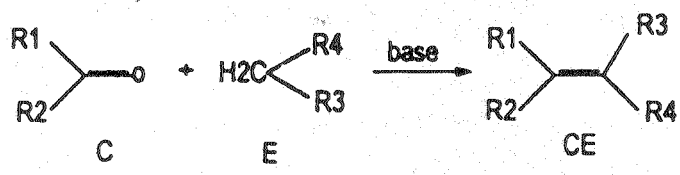
3-nitrophthalic anhydride, benzil, phthalimide and chloranil. The required compounds, 2,4 dinitro phenyl acetic acid, 3 nitrophthalic acid and benzil were synthesized employing reported procedures[10] while the remaining chemicals were purchased from Loba Chemie laboratory reagents and fine chemicals.

The library synthesis is based on simple and rapid nucleophilic condensation reactions[10] of carbonyl compounds (C_{1-4}) with active methylene compounds (E_{1-4}) to furnish combinatorial mixtures (C_nA_n) free of byproducts. Sixteen compounds were synthesized in two sets as eight combinatorial mixtures **(Scheme-1).**



Generation of Mixtures

Set1	Set2
E1.C1-4	C1.E1-4
E2.C1-4	C2.E1-4
E3.C1-4	C3.E1-4
E4.C1-4	C4.E1-4

CE	E1	E2	E3	E4
C1	C1E1	C1E2	C1E3	C1E4
C2	C2E1	C2E2	C2E3	C2E4
C3	C3E1	C3E2	C3E3	C3E4
C4	C4E1	C4E2	C4E3	C4E4

Scheme-1. Synthesis of the combinatorial minilibrary

In the first set each pure carbonyl compound (C_{1-4}) was reacted with stoichiometric amount of equimolar mixture of active methylene agents (E_{1-4}) and in another set each pure active methylene agent (E_{1-4}) was reacted with a stoichiometric amount of equimolar mixture of carbonyl compounds (C_{1-4}). Although the reactivities of active methylene agents (E_{1-4}) and carbonyl compounds (C_{1-4}) are different, the reaction conditions employed for the synthesis of a combinatorial library were adequate for quantitative conversion (TLC, 24 h) of a mixture of carbonyl compounds and active methylene agents to obtain a diverse menu of aryl and benzoyl substituted ethene derivatives for biological screening. The TLC analysis revealed that the concentrations of anticipated components in combinatorial mixtures are almost in the same proportion.

TABLE-1
ANTIMICROBIAL SCREENING OF LEAD COMPOUND

Organism	C_2E_3 (mm)	C_4E_3 (mm)	Streptomycin (mm)
	Zone of inhibition		
Bacillus subtilis	33	39	39
Staphylococcus aureus	36	19	39
Escherichia coli	27	19	24
Pseudomonas aeroginosa	33	19	29

Concentration of C_2E_3 and C_4E_3 = 500 mcg/mL
Concentration of streptomycin (ambistrin-S vial of 1 g) = 10 mcg/mL

Method for library preparation

0.4 M stock solutions of individual reactants (C and E) were prepared in methanol (20 mL). Solutions (10 mL) of all components from same reactant, *i.e.*, carbonyl compounds (C_{1-4}) and active methylene agents (E_{1-4}) were mixed separately to obtain C_{1-4} and E_{1-4}. In case of active methylene agents the solution of mixed components E_{1-4} was diluted to 40 mL with methanol to get 0.1 M of each reactant in solution. Solution (10 mL, 0.4 M) of individual reactant (C or E) and solution of mixed components of other reactants (C_{1-4} or E_{1-4}, 0.1 M, 10 mL) were mixed and aq. NaOH was added (2 M, 2 mL). The reaction mixtures were stirred continuously for 24 h, then concentrated to dryness *in vacuo*, neutralized with 1 N hydrochloric acid and extracted with $CHCl_3$ (2 × 40 mL). The combined organic layers were washed with water, brine and dried over anhydrous Na_2SO_4; concentration *in vacuo* furnished gummy or solid products, in quantitative yield.

TLC Analysis: The combinatorial mixtures C_{1-4} and E_{1-4} were analyzed by TLC. The TLC of combinatorial library mixtures was compared with the authentic mixtures prepared by mixing equimolar amounts of the compounds synthesized individually (C_4E_3, C_2E_3, C_1E_4, C_4E_2). They showed identical chromatographic profile.

Biological Evaluation: The combinatorial mixtures were subjected to antimicrobial assay using the cup-plate method[11]. The organisms used were obtained

from the stock culture of the Department of Pharmaceutical Microbiology in the National Chemical Laboratory, Pune, India. The organisms included *Staphylococcus aureus* ATCC 13709 (Sa), *Escherichia coli* ATCC 9637 (Ec), *Pseudomonas aeruginosa* ATCC 7853 (Ps), clinical isolate and *Bacillus subtilis* (Bs). Concentrations of these organisms were prepared to contain approximately 1×10^6 cfu/mL. 20 mL of nutrient agar was dispensed into sterile universal bottles, mixed gently and poured into sterile petri dishes. These were then inoculated with 0.2 mL of the cultures. After selecting, a cupbore with 9 mm diameter, it was properly disinfected with alcohol and sterilized by flaming and used to make six uniform cups in each petri dish. The cups were filled with the different freshly prepared solutions, 500 mcg/mL and allowed to diffuse for 45 min. The solvents [water/dimethyl sulphoxide] used were similarly analyzed. The plates were incubated at 37°C for 24 h.

Library Screening: The library mixtures were screened using the method described in biological evaluation. Two combinatorial mixtures (E_3C_{1-4} and C_2E_{1-4}), one from each set, gave highest antimicrobial activity in that set (19 and 29 mm at 500 mcg/mL concentration respectively), whereas the other mixtures gave a relatively insignificant response as compared to the standard inhibitor, streptomycin (ambistrin-S vial 1 g manufactured by Sarabhai Piramal Pharmaceutical Pvt. Ltd.). Considerable antibacterial activity of the combinatorial mixtures E_3C_{1-4} and C_2E_{1-4} indicated 1-naphthyl-2-(2-carboxy 3-nitrophenyl) ethene (C_2E_3) as expected in lead compound.

RESULTS AND DISCUSSION

The lead compound, 1-naphthyl-2-(2-carboxy 3-nitrophenyl) ethene (C_2E_3) was synthesized by condensing 3-nitrophthalic anhydride with 1-naphthyl acetic acid, which on evaluation for antimicrobial assay showed 29 mm zone of inhibition at 500 mcg/mL concentration.

For synthesis of 1-naphthyl-2-(2-carboxy 3-nitrophenyl) ethene, aq. NaOH (40 mmol, 4 mL) was added to a stirred solution of 3-nitrophthalic anhydride (1.93 g, 10 mmol) and 1-naphthyl acetic acid (1.86 g, 10 mmol) in methanol (10 mL). The reaction mixture was stirred at room temperature for 24 h. It was concentrated to dryness *in vacuo*, neutralized with hydrochloric acid and extracted with $CHCl_3$ (3×25 mL). The combined organic layer was washed with water, brine and dried over Na_2SO_4 and concentration of organic layer *in vacuo* followed by silica gel column chromatographic purification of the residue furnished pure C_2E_3 in quantitative yield with melting point 64°C.

IR absorption due to the (C=O) and (C=C) (conjugated) functions appeared at 1722 and 1576 cm^{-1}, respectively. Nitro group asymmetric and symmetric stretching at 1423 and 1308, respectively. In the aromatic region ^{1}H-NMR spectra shows three multiplets at 7.22 (3H), 7.37 (4H) and 8.25 (3H) and two duplets at 7.90 (1H) and 8.02 (1H).

In the course of optimizing reaction conditions the condensation product of chloranil with naphthyl acetic acid was synthesized, purified and screened by analogous method and the melting point of the product was found to be 78°C.

The product 2,3,5,6-tetrachloro-4-naphthalene-2-ylmethylene-cyclohexa-2,5-dienone 2,3,5,6-tetrachloro-4-naphthalene-2ylmethylene-cyclohexa-2,5-dienone (C_4E_3) was found to have greater activity (39 mm against *B. subtilis*) but had negligible activity against gram negative species used for evaluation.

IR absorption due to the (C=O) and (C=C) (conjugated) functions appeared at 1637 and 1572 cm^{-1}, respectively. Chloro group stretching at 1016 and naphthyl C—H at 787 cm^{-1}. In the aromatic region ^{1}H-NMR spectra show two multiplets at 7.20 (3H) and 7.32 (4H). The vinyl proton shows singlet at 7.68 (1H).

Conclusion

The results of this work suggest that manipulation of substituents, position of substituents and derivatization of functional groups may provide highly potent antimicrobials and further studies are in progress. In summary, we have synthesized a mini library of aryl and benzoyl substituted ethene derivatives by solution phase combinatorial chemistry and the biological evaluation results have provided a lead compound 1-naphthyl-2-(2-carboxy-3-nitrophenyl) ethene (C_2E_3) for further exploration to obtain a potent antimicrobial agent.

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